Mosher Declaration

APPENDIX 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	90/007,832
Confirmation No.	:	2201
Applicant	:	6573369
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Title	:	METHOD AND APPARATUS FOR SOLID STATE MOLECULAR ANALYSIS
TC/A.U.	:	3991
Examiner	:	Padmashri Ponnaluri
Docket No.	:	016348-9066-US00
Customer No.	:	023510

I, Monique Dillamon, hereby certify that this correspondence is being sent via Facsimile to Mail Stop Ex-Parte Reexam, Central Reexamination Unit, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date of my signature.

Monique Dillamon
Signature

May 1, 2007
Date of Signature

Mail Stop "Ex Parte Reexam"
Central Reexamination Unit
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Curtis Mosher, hereby declare and state the following:

1. I am currently the Vice President of Research and Development at BioForce Nanosciences, Inc., the owner of the above-referenced patent.

2. I received a Ph.D. in Biotechnology from Iowa State University of Ames, Iowa, in 1998, and a B.A. in Biotechnology in 1993 from the University of Northern Iowa in Cedar Falls, Iowa.

3. Attached as Exhibit A and incorporated herein by reference is a copy of my curriculum vitae.

4. I am a joint inventor of the invention described and claimed on this application.

5. I make this declaration in support of the prosecution of U.S. Reexamination Application Serial No. 90/007,832, which claims priority to U.S. Provisional Application No. 60/135,290, filed May 21, 1999 ("the May 1999 provisional").

6. I understand that the claims have been rejected under 35 U.S.C. § 103(a) as being obvious over Jones et al. (Analytical Chemistry, 70(7):1233-1241; "the Jones reference"), in

view of the January 7, 1999 provisional application of Mirkin et al., Appl. No. 60/115,133 ("the Mirkin provisional").

7. The Mirkin provisional describes the transfer of small amounts of synthetic organic molecules, such as alkanethiols, from an atomic force microscope (AFM) tip to a substrate, such as mica, in a controlled fashion to make a desired pattern. Each of the molecules deposited in the Mirkin provisional could withstand prolonged exposure to ambient air without adverse effects. Importantly, the Mirkin provisional does not describe the deposition of substances, such as proteins, that must be maintained in an aqueous solution for their stability.

8. The Jones reference describes the use of photolithography to generate patterned arrays having protein domains with a size of about 7.5 microns (Jones p. 1236, col. 2, lines 9-10 & Fig. 1B). Jones does not provide guidance for how submicron domains of proteins may be created. The photolithographic patterning techniques of Jones are unrelated to techniques utilizing AFM for deposition.

9. It was well known at the time of filing our May 1999 provisional application that proteins readily adhere to an AFM tip. Attached as Exhibit B (Rief et al. (1997) Science 276:1109-1111) and Exhibit C (Gergely et al. (2000) Proc. Natl. Acad. Sci. USA 97(20):10802-10807) are two references published before and after the filing date of the present application, respectively. At the time the May 1999 application was filed, proteins, when contacted with an AFM tip, were known to stick to the tip, and would remain stuck during use of the tip in AFM. See Exhibit B, Rief et al. at p. 1109, col. 2 line 11 through col. 3, line 1 (Si_3N_4 AFM tip binds to titin protein); see Exhibit C, Gergely et al. at p. 10803, col. 2, second sentence of third paragraph under "Materials and Methods" (fibrinogen protein adsorbed to Si_3N_4 AFM tip); see also Gergely et al. at p. 10804, col. 1, first full paragraph (proteins bound to Si_3N_4 AFM tip stick to the tip and not the substrate). The Jones reference and the Mirkin provisional do not provide guidance for how to overcome the problem of protein adherence to the tip.

10. Under my supervision, we recently attempted to fabricate nanoarrays of proteins using the AFM technique described by the Mirkin provisional. Since the Mirkin provisional provides no guidance as to the transfer of proteins, we used a saline buffer as the transport medium. However, we saw no evidence of protein transfer to the substrate. Rather, we found that the proteins remained associated with the AFM tip, and we were unable to transfer any protein in sub-micron domains from the tip to the substrate. In comparison, nanoarrays of proteins could

be successfully fabricated using the techniques described in our May 1999 provisional. The details of these experiments follow:

Standard silicon nitride AFM probes with tips were contacted with IgG containing a fluorescent label. Experiments were performed at 50% relative humidity. Dwell time for spotting of solution with the AFM probe onto the substrate was approximately 200ms.

1. Solution 1, as described in the May 1999 provisional, contained: 1mg/mL donkey anti-rabbit IgG labeled with Alexa Fluor 488 (from Molecular Probes #A11055), 0.5 x PBS, 50mM NaP (sodium phosphate), 50mM NaCl, 2.5mM azide, 5% Glycerol.
2. Solution 2 contained: 1mg/mL donkey anti-rabbit IgG labeled with Alexa Fluor 488 (from Molecular Probes #A11055); 0.5 x PBS; 50mM NaP; 50mM NaCl; 2.5mM azide.
3. 0.5 microLiters of solution 1 was placed on a coverslip.
4. AFM tip and cantilever was dipped into the drop for 10 sec.
5. AFM tip was removed.
6. AFM tip was lowered into contact with APTES (3-Aminopropyltriethoxysilane)-coated glass coverslip at a predetermined point.
7. The tip was raised and a submicron sized spot of deposited material could be seen.
8. Steps 6 and 7 were repeated several times in a horizontal line pattern.
9. 0.5 microLiters of solution 2 was placed on a second coverslip.
10. Steps 4-8 were repeated with a new AFM probe
11. Each of the two patterned coverslips were taken to a fluorescence microscope and viewed in both brightfield and epifluorescence.
12. Spots visible in brightfield show where the tip contacted the substrate. However, spots are visible in epifluorescence only when the labeled protein is present in the domain.

The results of these Experiments are shown in Exhibit D (attached). The size of each spot in each of the panels was 600-900 nm. The images taken in the brightfield show the position of spots where the tip contacted the substrate when either solution 1 (Panel A) or solution 2 (Panel C) was used. Three spots are evident in Panel A, and two in Panel C. No sub-micron fluorescent spots (Panel D) corresponding to the position where the tip was contacted with the

substrate (Panel C) were noted under epifluorescence when solution 2 was used. Any diffuse background fluorescence in Panel D does not correlate with the brightfield spots shown in Panel C. Therefore the sub-micron sized spots shown under brightfield in Panel C from the deposition of solution 2 did not result in sub-micron sized protein domains. In contrast, fluorescence of the spots was noted under epifluorescence in Panel B when solution 1, as described in the May 1999 provisional, was used. The spots in Panel B correspond to the position where the tip contacted the substrate of the protein-containing in submicron domains in Panel A. Therefore, protein was directly deposited onto the surface from the tip when solution 1 was used.

11. The results of the experiment using solution 2 mirror our initial attempts to transfer protein solutions from an AFM tip to a substrate prior to the filing of the May 1999 provisional. In these experiments, it appeared that only the carrier solution was transferred to the substrate, whereas the protein remained associated with the AFM tip. However, we discovered that by using glycerol in the solution, proteins could be effectively transferred in sub-micron domains, along with the carrier solution, to the substrate, and the presently claimed nanoarrays of protein could be produced.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I further declare that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: May 1, 2007

Curtis Mosher